

# The shifting balance of facilitation and competition affects the outcome of intra- and interspecific interactions over the life history of California grassland annuals

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**Abstract** Trait-based resource competition in plants, wherein more similar plants compete more strongly for resources, is a foundation of niche-based explanations for the maintenance of diversity in plant communities. Alternatively, neutral theory predicts that community diversity can be maintained despite equivalent resource requirements among species. We examined interactions at three life history stages (germination, survival, and juvenile-adult growth) for three native and three exotic California annual species in a glasshouse experiment. We varied plant density and species composition in small pots, with pots planted with either intraspecific seeds or in a three species mix of intra- and interspecific neighbors. We saw a range of facilitative, neutral, and competitive interactions that varied significantly by species, rather than by native or exotic status. There were more competitive interactions at the emergence and juvenile-adult growth stages and more facilitative interactions for survival. Consequently, the relative

strength of competition in intraspecific versus mixed-species communities depended on whether we considered only the juvenile-adult growth stage or the entire life history of the interacting plants. Using traditional analysis of juvenile-adult growth only, all species showed negative density-dependent interactions for final biomass production. However, when the net effect of plant interactions from seed to adult was considered, which is a prediction of population growth, two native species ceased to show negative density dependence, and the difference between intraspecific and mixed-species competition was only significant for one exotic species. Results were consistent with predictions of neutral, rather than niche, theory for five of six species.

**Keywords** Facilitation · Interspecific competition · Intraspecific competition · Annual grasslands · Seedling · Niche · Neutrality

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## Introduction

All plants have the same requirements for growth: light, water, and nutrients. Species differ in their ability to sequester resources and/or grow and reproduce at lower resource levels (Tilman 1982), leading to the hypothesis that competitive interactions should be a primary influence on plant community composition (Goldberg and Barton 1992). However, it is

increasingly recognized that facilitation may also be important in plant growth and establishment, and that the balance between competition and facilitation can change over the lifespan of plants (Callaway and Walker 1997; Armas and Pugnaire 2005; Schiffrers and Tielborger 2006; Lortie and Turkington 2008). For example, high densities of seeds can inhibit germination (negative density dependence), promote survival (positive density dependence), and decrease overall plant size (negative density dependence), all in the same community of interacting plants (Goldberg et al. 2001). While plant–plant interactions, both positive and negative, are important, the combined impact of these interactions on seed production and thus population growth has rarely been assessed in manipulative experiments.

Facilitative plant–plant interactions have been examined in a burgeoning number of studies, most often focusing on interspecific interactions between older plants and newly established seedlings (reviewed in Brooker et al. 2008). Facilitation is predicted to be greatest between plants in different functional groups or at different life history stages because they differ in their immediate resource needs (Callaway 1995; Callaway and Walker 1997; Brooker et al. 2008). Many reviews omit or have very short discussions of positive interactions between plants at the same life history stage (e.g., Callaway and Walker 1997; Brooker et al. 2008). However, one of the most predictably positive, density-dependent responses in plants is a “group advantage” in seedling establishment (Bertness and Yeh 1994; Bruno et al. 2003). Positive, density-dependent survival among seedlings in single-species or multi-species mixes of the same functional group is not uncommon (Shaw and Antonovics 1986; Bertness and Yeh 1994; Goldberg et al. 2001; Humphrey and Schupp 2004; Skinner 2005; Schiffrers and Tielborger 2006; Lortie and Turkington 2008). A full description of the strength of plant interactions should include all the life history stages of plants from seed germination to seed production.

Despite the potential for facilitative interactions to occur at early life history stages, outcomes of plant–plant interactions are usually described as the result of a plant’s ability to translate resources into growth in later life history stages: the effect of density on average plant size is usually calculated by measuring changes in average plant biomass or fecundity by the

density of surviving plants, omitting seeds that have failed to germinate or seedlings that have died (e.g., Goldberg et al. 2001). Including the net effects of density on seed germination, survival, and growth, rather than just focusing on growth, could influence the outcome of competition studies, and can change our expectations about which plants will dominate a given community (Tilman 1990; Goldberg et al. 2001; Lortie and Turkington 2002; Adler et al. 2007).

Considering the net effects of plant interactions may also affect the conclusions of experiments designed to test the relative importance of niche and neutral theories for the maintenance of community diversity. Historically, most efforts to explain plant species coexistence have been based on a niche-based theoretical framework. Within this framework, differences in the timing and type of resource capture among plant species allow for their coexistence (MacArthur and Levins 1967). Niche theory is an evolutionary perspective on plant competition: it is through evolution that we expect plants to come to exploit resource bases that differ from one another (Holt and Gaines 1992). If individuals of the same species have more similar resource use than members of different species, then intraspecific competition would be predicted to be stronger than interspecific competition. Thus, species coexistence is promoted through stabilizing, or negative density-dependent, interactions, wherein species limit their own growth more than that of other species, and community diversity is maintained (MacArthur and Levins 1967; Antonovics and Levin 1980; Adler 2004; Silvertown 2004; Harpole and Tilman 2006). Alternately, neutral theory maintains that random differences in dispersal, death, and establishment are sufficient to retain community diversity, and predicts no consistent difference in the strength of intra versus interspecific competition between interacting species (Hubbell 2005).

Comparing the performance of individual species when they experience primarily intraspecific versus primarily interspecific interactions can indicate the relative importance of niche versus neutral forces for maintaining diversity in particular communities. In short-lived organisms, one can measure the outcome of experimental interactions directly by measuring population growth, and infer that negative density dependence maintains community diversity if populations grow more slowly when they are common than when they are rare (e.g., Zhang et al. 2009).

Most experimental plant studies measure only the outcome of one generation of interactions, and infer the importance of niche versus neutral processes for maintaining diversity based on the relative fitness of plants grown with intra versus interspecific competition (e.g., Harpole and Suding 2007). Reviews of the relative strength of intra and interspecific competition in experimental plant studies tend to support neutral theory, finding little evidence for consistently stronger intraspecific competition in interacting species (Goldberg and Barton 1992; Gurevitch et al. 1992). Results from more recent studies not included in these two reviews also demonstrate variable responses, with some species showing stronger intraspecific competition (Morishita et al. 1991; Gordon and Rice 1992; Sheley and Larson 1994; Shrefler et al. 1994; Velagala et al. 1997; Clausnitzer et al. 1999; Harpole and Suding 2007; Wassmuth et al. 2009) and others showing no difference or stronger interspecific competition (Gordon and Rice 1992; Pantastico-Caldas and Venable 1993; Shrefler et al. 1994; Clausnitzer et al. 1999; Aguiar et al. 2001; Schmidt et al. 2008; Powell and Knight 2009; Wassmuth et al. 2009). However, as is customary in plant competition studies, all of these experiments were conducted on the growth-only stages and, therefore, may not be appropriate for inferring the importance of density dependence for maintaining community diversity.

Finally, the evolutionary history of a competing group of plants may affect the strength of plant–plant interactions (Turkington and Harper 1979; Martin and Harding 1981; Evans et al. 1985; Aarssen and Turkington 1985; Espeland and Rice 2007; Wilsey et al. 2009). If niche differentiation is important for maintaining community diversity, one would expect that species that have co-occurred for longer periods of time would have greater niche separation than species with a shorter coevolutionary history. That is, if interactions between populations lead to a continuing evolution of niche differentiation, then one would expect to see evidence of stronger interspecific competition in recently assembled plant communities (i.e., among exotic species), because there has been a much shorter time frame for natural selection to favor the partitioning of resource use. Wilsey et al. (2009) provide support for this hypothesis with experimental communities of recently assembled, exotic species and potentially coevolved native ones, and observe

greater maintenance of diversity in native communities. This is consistent with the hypothesis that stabilizing interactions are more common within native communities, as niches become differentiated through evolutionary time.

This experiment examined plant–plant interactions among historically co-occurring species and among species that are recent arrivals in the California annual grassland. First, we examined the type of density-dependent interactions (positive/facilitative, neutral, or negative/competitive) within intraspecific mixes across three life history stages (germination, survival, and growth) of six annual plant species grown at low and high densities. Second, we compared results from these intraspecific interactions with the strength of competition for each of the six species grown as targets in a mixed-species community, where a target plant experienced a mix of interactions with conspecifics and heterospecifics. Next, we determined whether there were differences in the strength or direction of plant–plant interactions among historically co-occurring native species compared to recently introduced exotic species. Finally, we asked whether including the net effects of plant interactions at three life history stages changes conclusions about the relative importance of negative density dependence and differences between intra- and interspecific competition. Stabilizing interactions (evidenced by stronger competition in monospecific communities than in mixed-species communities) and stronger interactions between interacting exotic species would be evidence for a niche-based model of community assembly. We expected that an analysis that included the entire life history, including potential facilitation in early stages, might decrease the strength of competition between interacting plants, as well as affect conclusions about the processes that dominate this community.

## Methods

Field-collected seeds from six co-occurring California grassland annuals were planted into a native field soil (a sandy clay loam) in 2 cm × 3 cm × 10 cm deep pots on 13 Nov 2006. The soil was not treated to kill the resident seed bank, but the surface area of the pots was small and the germinable soil fraction was low; therefore, there was limited opportunity for nonplanted seeds to emerge. Seeds were collected at

multiple locations within the Donald and Sylvia McLaughlin Reserve (University of California Natural Reserve System) at the intersection of Lake and Napa counties in California, 38° 52' 26" N latitude, 122° 25' 54" W longitude. Five field soil samples (collected from the top 10 cm) were sent to A & L Western Agricultural Laboratories, Modesto, CA, for analysis of texture and nutrient content. This reserve contains many serpentine and serpentine-influenced soils and, thus, has a relatively high proportion of native species, relative to other California grasslands. Commonly found species with contrasting growth forms were selected. The three native and three exotic species were: *Lasthenia californica* Lindley (native forb), *Plantago erecta* Morris (native forb), *Vulpia microstachys* (Nutt.) Munro (native grass), *Erodium brachycarpum* (Godr.) Thell. (exotic forb), *Geranium dissectum* L. (exotic forb), and *Bromus madritensis* subsp. *rubens* (L.) (exotic grass). The exotic annuals are naturalized, but not highly invasive in this location, and the rank abundance of each species on the landscape, from the most to least common, is *P. erecta*, *V. microstachys*, *L. californica*, *B. madritensis*, *E. brachycarpum*, and *G. dissectum* (S.P. Harrison, unpublished data). We chose a mix of forbs and grasses to maximize potential diversity of root structures, as forbs can be tap-rooted or have relatively thick roots, while grasses typically produce fine roots that explore soil space quite differently (e.g., Craine et al. 2001). In addition, the selected forbs have different above-ground growth habits, as rosette-formers (*P. erecta*, *E. brachycarpum*) and as those that grow more erect (*L. californica*, *G. dissectum*). Seeds were mixed equally among the collection locations, and grown in a field-collected soil from the reserve. Initially, the experiment was planned to include an investigation into the effects of mycorrhizal infection on plant interactions, and half of the pots received a fungicide treatment (Iprodione, 2 g/m<sup>2</sup>) every 2 weeks starting on 12 Nov 2006. However, the fungicide treatment had only minimal influence on plant survival (control = 81.2% ± 0.3% SE, fungicide = 88.8% ± 0.4% SE,  $P = 0.02$ ,  $R^2 = 0.01$ ), no effect on plant growth ( $F_{1, 312} = 0.15$ ,  $P = 0.70$ ), and neither increased nor decreased facilitation or competition ( $F_{2, 312} = 0.33$ ,  $P = 0.72$ ). This was likely due to high soil phosphorus levels (Grant et al. 2004), and hereafter, this treatment is ignored in analyses.

Pots were arranged in 20 trays for ease of watering, and each tray functioned as a block, containing one replicate of three different density treatments for each of the six species. Each species was sown at low intraspecific densities (3 seeds), high intraspecific densities (8 seeds), and each species sown as the target species within a high-density intra- and interspecific mix (8 seeds). Sown densities represented 5,000 seeds/m<sup>2</sup> (low density) or 13,000 seeds/m<sup>2</sup> (high density). The low-density treatment is in the range of expected densities of California annual grasslands, which have on the order of 4,125 stems/m<sup>2</sup> (Zavaleta and Hulvey 2004). The most common species in this study area, *Plantago erecta*, has been observed to have densities as high as 5,600 plants/m<sup>2</sup> in the populations from which our seeds were collected (Espeland and Rice in press). High-density mixes were planted in a replacement design, keeping seeding density constant but changing the identity of competitors. Species mixes were either entirely native or entirely exotic, with each species represented as a target species (4 seeds) and the other two native or exotic species, as appropriate, sown as competitor species (2 seeds of each). Our planted intraspecific densities ranged from 3 seeds (low density, intraspecific), to four seeds (high density, interspecific), to eight seeds (high density, intraspecific); thus we would expect results for the interspecific treatment to be intermediate to both high- and low-density single-species pots if interactions between intraspecific plants are stronger than with interspecifics.

We chose to measure the relative strength of interspecific interactions by comparing plant performance in intraspecific-only mixes with a mixed-species community that includes both intra- and interspecific competition, rather than with a single interspecific competitor. The drawback of our method is that it does not provide the exact magnitude of interspecific competition in our three species mixes (which would require pairwise comparisons of all the three interacting species). However, knowing the strength of pairwise interactions would not tell us how those interactions would affect target plant fitness in a mixed-species community. Natural communities include a mix of interactions with conspecifics and multiple other species; hence we sacrifice some precision for a more relevant result.

Plants were grown in the greenhouse at the University of Nevada, Reno. Block locations were

re-randomized every 2 weeks, and plants were watered 1–2 times a week. Seedling emergence and survival were monitored every 1–4 days until 10 Jan 2007, with more frequent monitoring in initial weeks and less frequent monitoring as birth and death rates neared zero. Emergence was recorded when a cotyledon was visible above the soil surface, and any plant that died prior to harvest was recorded as dead. Harvesting occurred after 5 months, when some of the species were becoming senescent. Blocks were harvested sequentially, with each entire block harvested on the same date. Above-ground biomass of each plant was harvested separately and dried at 60°C until it reached a constant weight. We separately weighed both the entire above-ground biomass and the reproductive biomass for each plant. As the phenology of each species was slightly different, reproductive biomass ranged from mature seeds (*E. brachycarpum*, *V. microstachys*, *P. erecta*) to immature seeds (*B. madritensis*) to flowers (*L. californica*), or flower buds (*G. dissectum*). Owing to this, we used vegetative biomass as our dependent fitness-related variable instead of reproductive biomass, as it is a measure of the potential fitness of individual annual plants irrespective of plant phenology at the time of harvest. There was a high degree of correlation between reproductive structures and plant biomass for all species ( $R^2$  values of  $0.82 \pm .06$  SE, all  $P < 0.0001$ ).

#### Data analysis

All the analyses were conducted with JMP 5.0.1 (SAS Institute, Cary, North Carolina), and all the values are presented as means  $\pm$  standard error (SE). We analyzed both the effect of planting density on seed germination and emerged seedling density on survival using logistic regression, with emergence or survival (0/1) of each plant in each pot as a response variable. These models included the following factors: block (random factor), status (fixed factor, either native or exotic), species (random factor, nested within status), and pot density (fixed factor) as well as two-way interactions between status, species, and density, with categorical survival/death for each plant in each pot as the response variable. Pot densities were analyzed as categorical treatments, designated as either high-density single-species (eight seeds, or > four seedlings), high-density mixed-species

(eight seeds, or > four seedlings), or low-density single-species (three seeds, or two or three seedlings) based on either the number of seeds planted, or the number of seedlings survived, depending upon whether the analysis measured net interactions (seed to adult interactions) or growth only (juvenile to adult interactions only), explained in detail below. The density of seeds planted per pot was used to analyze differences in germination, and the density of seeds emerged per pot was used to analyze survival. Owing to lack of replication in high-density pots, *L. californica* was not included in survival analysis, though results are displayed graphically. In mixed-species pots, only the germination and survival of the target plants were considered in the analysis. ANOVA was used to compare the density of plants per pot at the end of the experiment using this same model structure, with plant density as a response variable.

Final plant biomass was analyzed in two different ways. The first analysis evaluated the interactions between plants that survived to adulthood, and excluded seeds that failed to germinate and plants that died. Biomass values were log-transformed to meet assumptions of ANOVA, using the same model statement described above. Again, one native species, *L. californica*, had to be excluded from analysis of single-species pots and as a target species in mixed-species pots, because of poor germination and lack of replication for high-density treatments, but data are presented in graphical form. *Lasthenia californica* was included in the analysis described below, however.

In the second analysis, biomass values were averaged for all individuals within pots, including zero values for seeds that did not germinate and seedlings that died. Analyses were conducted on this untransformed, average value, rather than on individual plant weights. This method allowed for calculation of the expected biomass production per seed planted in a pot while minimizing the problem of multiple 0 values in a data set violating the assumptions of ANOVA. This analysis represents the net effects of facilitation, neutral, or competitive interactions in each pot, for all life history stages. As species by density interactions were significant for both biomass analyses, ANOVA was conducted separately for each species and Tukey HSD comparisons of the three density treatments were made (model effects were block, random factor, and density). Only target plants in

mixed-species pots were included in analyses of plant size.

A relative interaction index (RII) was calculated for intraspecific pots and mixed pots for all six species at each life history stage and for each measure of plant growth. The RII was calculated as  $(\text{Average}_H - \text{Average}_L)/(\text{Average}_H + \text{Average}_L)$ , where the average represents overall values for each species at high (H) and low (L) densities, for each life history stage. We used an average value, rather than the customary method of calculating a difference between paired experimental units (Armas et al. 2004), because paired samples were only available for two of our four response variables (measures of germination and net biomass) due to variable germination and survival of treatments in each block. Thus, there is no measure of error associated with each RII value, and we performed no statistical analyses of the index. Results of this calculation are presented to visualize effect sizes of plant interactions, with significance measures displayed from the results of the ANOVA and logistic regressions described above.

## Results

### Soil analysis

The field soil was a sandy clay loam, with  $49.0 \pm 1.5\%$  sand,  $25.4 \pm 1.3\%$  silt, and  $25.2 \pm 0.6\%$  clay, with a medium to high organic content ( $3.7 \pm 0.4$ ), very low N ( $\text{NO}_3$  4 ppm  $\pm$  0), high to very high P ( $28.2 \pm 1.2$  ppm extracted with a weak Bray method), and medium levels of K ( $172.2 \pm 9.2$  ppm). As is customary in these serpentine soils, Mg was very high ( $1115.6 \pm 21.2$  ppm) and Ca was low ( $1923.6 \pm 21.2$ ).

### Emergence

Of the 2,280 seeds sown, overall emergence was 71.1%. Emergence was significantly higher for exotic species (Table 1A), with  $88.2 \pm 1.6\%$  of seeds emerging per block, compared to  $54.0 \pm 3.0\%$  emergence of native seeds. Species differed significantly in their emergence rates (Table 1A). Owing to either low seed viability or seed dormancy, *Lasthenia californica* had extremely low emergence of only  $27.9 \pm 3.2\%$ . This was the case in both single-species and mixed-species

**Table 1** Probability of germination (A) and survival of emerged seedlings (B)

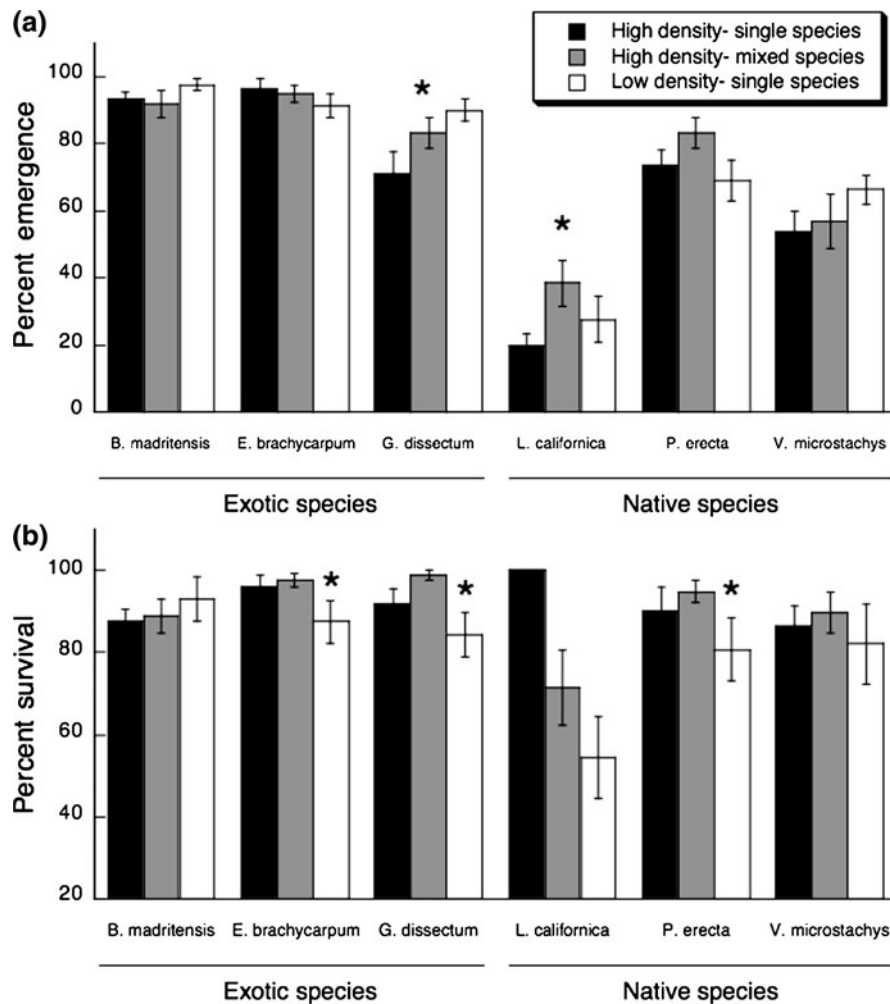
Factor	A. Germination		B. Survival	
	$\chi^2_{[df]}$	<i>P</i>	$\chi^2_{[df]}$	<i>P</i>
Block	54.05 <sub>[19]</sub>	<b>&lt;0.0001</b>	31.76 <sub>[19]</sub>	<b>0.0332</b>
Status	181.38 <sub>[1]</sub>	<b>&lt;0.0001</b>	6.46 <sub>[1]</sub>	<b>0.0110</b>
Species (status)	134.43 <sub>[4]</sub>	<b>&lt;0.0001</b>	5.05 <sub>[3]</sub>	0.1680
Density	4.72 <sub>[4]</sub>	0.0945	16.81 <sub>[2]</sub>	<b>0.0002</b>
Status * Density	2.64 <sub>[2]</sub>	0.2668	3.02 <sub>[2]</sub>	0.2207
Species (status) * Density	20.55 <sub>[8]</sub>	<b>0.0085</b>	13.51 <sub>[6]</sub>	<b>0.0355</b>

Each treatment was represented once within each block, and germination was analyzed on a per-seed or seedling basis using logistic regression. The density treatment for the germination analysis is the density of planted seeds; the density treatment for the survival analysis is the density of seeds that germinated in each pot. Block and species are random effects; status and density are fixed factors. Bolded *P*-values highlight factors with significant ( $P < 0.05$ ) model effects; df indicates degrees of freedom

pots; therefore, *P. erecta* and *V. microstachys* were each other's most frequent neighbors in the native mixed-species pots. Overall emergence percentages of other species were  $60.5 \pm 3.2$  for *V. microstachys*,  $75.3 \pm 2.8$  for *P. erecta*,  $73.7 \pm 2.6$  for *G. dissectum*,  $92.6 \pm 1.5$  for *B. madritensis*, and  $94.2 \pm 2.1$  for *E. brachycarpum*. Owing to the low emergence of *L. californica*, this species never reached high enough densities to be present in more than one high-density treatment.

Planting density affected emergence differently for different species (Table 1A, significant species by planting density interaction, species-specific means shown in Fig. 1a). Two species, one exotic (*G. dissectum*) and one native (*L. californica*), showed signs of intraspecific inhibition of seed emergence. These seeds had the lowest emergence when planted at high densities in single-species pots (Fig. 1a). There were no significant effects of density on emergence for the remaining four of the six species (Fig. 1a). Timing of emergence was different among species ( $P < 0.0001$ , analysis not shown), with *B. madritensis* emerging  $5.3 \pm 1.1$  days after sowing, and with the timing of other species at  $3.7 \pm 1.1$  for *E. brachycarpum*,  $7.7 \pm 1.2$  for *V. microstachys*,  $9.6 \pm 1.1$  for *P. erecta*,  $13.4 \pm 1.4$  for *L. californica*, and  $17.6 \pm 1.1$  for *G. dissectum*. The presence of seeds of other species in the pot did not affect emergence timing (low-density emergence date





**Fig. 1** **a** Percent emergence in pots of different density and species composition. Planting in high-density, single-species pots consisted of eight seeds; high-density, mixed-species had four seeds of the target plant and two seeds each of two additional species; low-density pots were sown with three seeds of the target pot. Analyses were conducted with logistic regression, but percentages are displayed here for ease of viewing. There were significant affects (indicated with \*) of planting density on germination of two species, *G. dissectum*

similar to mix emergence date,  $P > 0.8$ , analysis not shown).

### Survival

Overall survival of emerged seedlings was high. Of 1,598 seedlings that emerged, 88.2% survived. Exotic species survived significantly better than native species (Table 1B), with  $91.0 \pm 1.5\%$  of emerged seedlings surviving compared with  $75.9 \pm 3.8\%$  of

( $\chi^2 = 13.71$ ,  $P = 0.0011$ ), *L. californica* ( $\chi^2 = 8.64$ ,  $P = 0.0133$ ). **b** Percent survival in pots of varying seedling density (density of seedlings that were observed emerging) and species composition. Seedling density affected survival for *E. brachycarpum* ( $\chi^2 = 11.12$ ,  $P = 0.0039$ ), *G. dissectum* ( $\chi^2 = 12.63$ ,  $P = 0.0018$ ), and *P. erecta* ( $\chi^2 = 11.03$ ,  $P = 0.0040$ ). Results for *L. californica* were not analyzed statistically because of poor replication, but results are presented for visual comparison

native species surviving after emergence. The emergence of seeds that germinated in a pot significantly affected probability of survival (Table 1B), and this differed significantly by species (significant species by density interaction, Table 1B, Fig. 1b). For three of the five species analyzed (*E. brachycarpum*, *G. dissectum*, *P. erecta*), there was significantly higher survival in high-density single-species and mixed-species pots than in low-density pots. This trend was also seen in *L. californica* (not analyzed because of

lack of replication in high-density pots) and *V. microstachys*, though not statistically significant. *Bromus madritensis* was the only species that showed an opposite, though nonsignificant trend.

### Biomass

Among plants surviving to adulthood, there was an overall effect of total pot density on plant size (Table 2A), as well as differential effects of density on plant size of all species (species  $\times$  density interaction, Table 2A, Fig. 2a, species-specific means shown in Fig. 2). All species showed significant decreases in average plant size when grown in single and mixed-species high-density pots, relative to plant sizes in single-species low-density pots (Fig. 2a). Two species, *B. madritensis* and *P. erecta*, responded similarly to intra- and mixed-species competition, showing the same decrease in plant size whether the competitors were entirely the same species or different species. Intraspecific competition was stronger than mixed-species competition for two species, *E. brachycarpum* and *V. microstachys*, with plants grown in mixed-species, high-density pots showing intermediate plant size to low- and high-density single-species pots. *Lasthenia californica* showed this same pattern, though results were not analyzed. Finally, *G. dissectum* showed significantly stronger effects of mixed-species competition, with the smallest plants grown in high-density, mixed-species pots.

When net growth was analyzed, which predicts population growth, rather than resource competition between surviving plants, these results changed. As germination and survival were not completely random

processes (Table 1, Fig. 1a, b), including 0 values in the data set did not simply depress mean values of all biomass measurements equally in all density treatments. Instead, there were both significant interactions between density and status and density and species (Table 2B, Figs. 2b, 3). With this method, intra- and mixed-species competition had similar effects on *B. madritensis*, *G. dissectum*, *L. californica*, *P. erecta*, and *V. microstachys*, and intraspecific competition was stronger than mixed-species competition only for *E. brachycarpum* (Fig. 2b). In contrast to the analysis of the strength of interactions between plants that survived to adulthood (Fig. 2a), there was no longer evidence for negative density dependence in *V. microstachys* (Fig. 2b).

The Relative Interaction Index summarizes the magnitude and direction of interactions across all three life history stages, comparing high density (both intra- and interspecific mixes) to the performance of plants grown in low densities (Fig. 3). The negative direction of most bars indicates the mostly negative interactions with increasing densities of interacting plants, with the exception of the plant survival stage. Differences between intra- and interspecific mixes are also apparent for three of the six species, as are the differences between analyses that consider only plants that survived until the end of the experiment and analyses that consider survival from seed (Fig. 3).

### Discussion

Competitive interactions are integral to theories of community coexistence, but plant–plant facilitation is generally omitted from coexistence models (but see

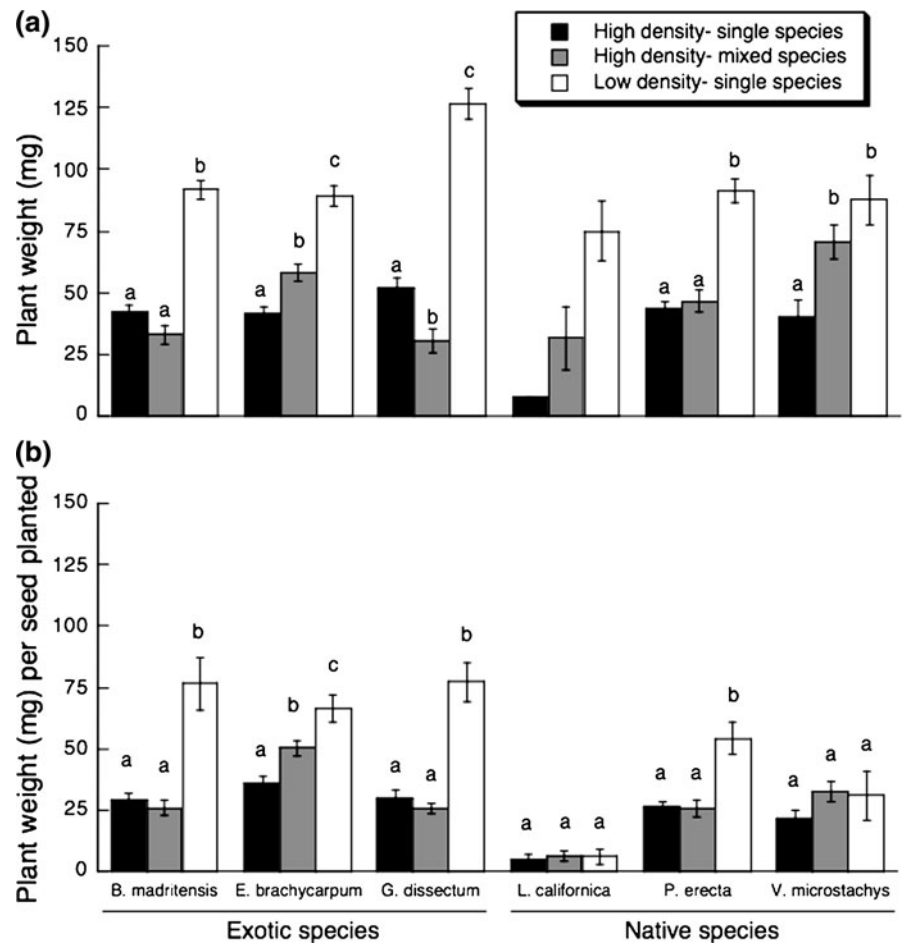
**Table 2** Plant biomass analyzed two different ways: only including plants that survived until the end of the experiment (A) and including zero values for seeds that either did not germinate, or germinated and died (B)

Factor	A. Biomass of surviving plants			B. Biomass produced per seed planted		
	df	F	P	df	F	P
Block	19, 1050	1.66	<b>0.0377</b>	19, 323	2.27	<b>0.0020</b>
Status	1, 3.3	0.53	0.5164	1, 4	6.24	0.0669
Species (status)	3, 6.1	0.97	0.4661	4, 8	4.10	<b>0.0425</b>
Density	2, 6.3	14.42	<b>0.0045</b>	2, 8	14.32	<b>0.0023</b>
Status * Density	2, 6.3	0.24	0.7909	2, 8	4.73	<b>0.0441</b>
Species (status) * Density	6, 1050	6.55	<b>&lt;0.0001</b>	8, 323	3.89	<b>0.0002</b>

In (A), the density treatment is the density of live plants in a pot at the end of the experiment, and in (B), density is the number of seeds planted per block. Block and species are random factors; status and density are fixed. Bolded *P*-values highlight factors with significant ( $P < 0.05$ ) model effects; degrees of freedom (df) are indicated as numerator df, denominator df



**Fig. 2** Average above-ground of plants that survived to the end of the growing period (a) and average biomass per seed planted (b), in high-density and low-density pots. Data shown are LSM from full model run on untransformed data, but analyses were conducted on log-transformed data. Data from *L. californica* is displayed, but was not analyzed due to small sample size. Letters indicate significant differences between treatments determined by Tukey's HSD tests, with comparisons made only within species



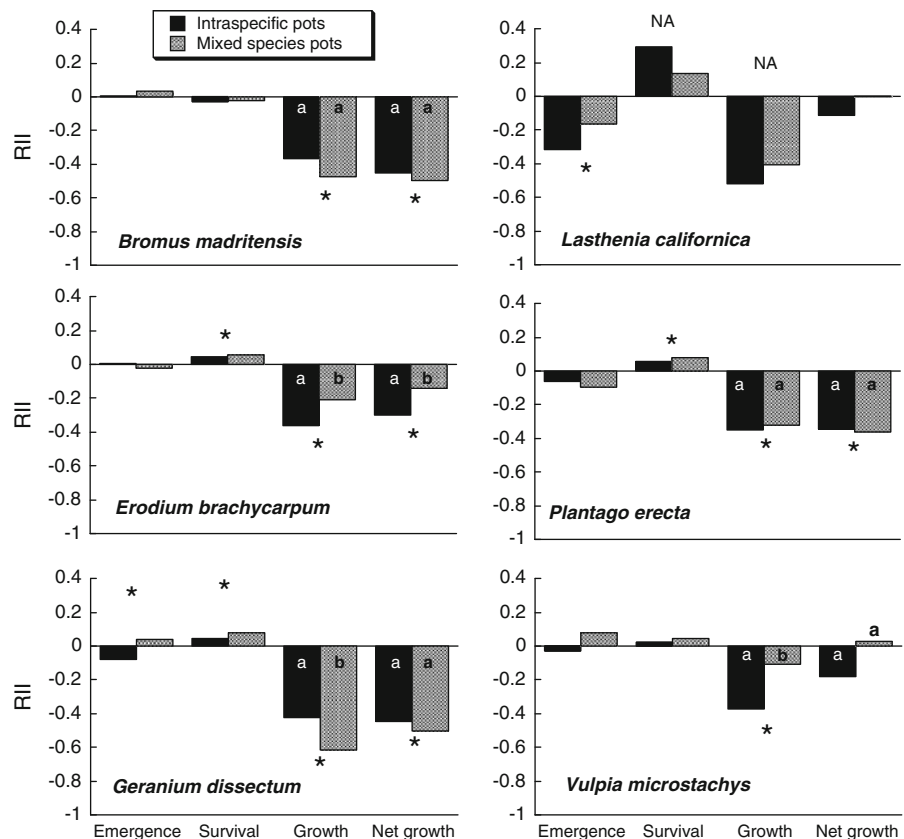
Travis et al. 2005, 2006; Vellend 2008). We observed a range of facilitative, neutral, and competitive interactions in this experiment, with competitive interactions seen for two species during germination and within all species in the plant growth stages, and three species showed facilitative interactions for survival. We found that support for the predictions of niche or neutral models was analysis-dependent, with either one or two of six species showing stabilizing density-dependent reductions in fitness. Most differences in plant interactions were species-specific and stage-specific unrelated to native or exotic status; however, the analytical method used affected this conclusion.

Two species in this study (one native and one exotic) showed negative density-dependent germination, with a greater suppression of germination under intraspecific, rather than mixed-species, competition. Results from other studies on the effect of seed density on germination within both intraspecific mixes (Linhart

1976; Shaw and Antonovics 1986) and interspecific mixes (Inouye 1980; Goldberg et al. 2001; Lortie and Turkington 2002; Shilo-Volin et al. 2005) have found a range of responses, from negative density dependence (Palmbad 1968; Linhart 1976; Inouye 1980; Goldberg et al. 2001; Lortie and Turkington 2002; Shilo-Volin et al. 2005) to neutral (Linhart 1976; Shaw and Antonovics 1986; Howard and Goldberg 2001) or positive density dependence (Linhart 1976; Howard and Goldberg 2001). Negative density dependence at the germination stage is a stabilizing process and in an analysis of the outcome of net plant interactions, magnifies the effects of density-dependent competition seen at the juvenile-adult growth stage.

In contrast, positive density dependence for survival can counteract negative density dependence at other stages. In our experiment, survival rates were either unaffected or improved with increasing seedling density, which has been seen in other studies

**Fig. 3** Relative Interaction Index (RII) calculated on overall average values presented in Figs. 1 and 2. The left three panels are exotic species, and the right three panels are native species. Analyses were not conducted on RII values, but presented to visualize the strength and direction of plant interactions. Positive values indicate facilitative interactions and negative values indicate competition. Significance is as shown in Figs. 1 and 2: a significant effect ( $P < 0.05$ ) of density on life history stage is indicated with \*, and additionally, for growth measures, letters indicate significant differences between intraspecific and mixed-species pots. “NA” indicates that survival and growth analyses were not conducted on *L. californica* (due to low sample sizes)



(Shaw and Antonovics 1986; Bertness and Yeh 1994; Goldberg et al. 2001; Humphrey and Schupp 2004; Skinner 2005; Schiffrers and Tielborger 2006; Lortie and Turkington 2008). Five of the six species studied here showed qualitatively higher survival at higher planting densities of both intraspecifics and mixed-species. There are many proposed mechanisms for facilitation between plants (reviewed in Callaway 1995; Hunter and Aarssen 1988). These include herbivore protection, increased availability of soil nutrients, and amelioration of light, temperature and moisture environments. All of these mechanisms, save herbivore protection (there were no herbivores in the greenhouse), could have increased survival of seedlings in this experiment. The self-thinning rule, whereby seedling survival decreases at increasingly high densities (Westoby 1984) did not apply. Though we planted at high densities, and plant sizes were considerably smaller in high-density pots, densities were not above the carrying capacity of the pots, as total pot biomass increased with increasing plant density for all species and species mixes (data

not shown). This was surprising, given that our low-density plantings were in small pots, in field soil, at the maximum field-observed densities of these plants at their seed collection location. We surmise that greenhouse water application, though not liberal, may have been the factor that increased the carrying capacity of these pots.

As a result of the effects of neighbors on germination and survival, the conclusions from a traditional growth analysis (juvenile to adult stages only) differed from an analysis of net growth (including seed and seedling interactions). Using traditional analysis, there was negative density dependence in plant growth for all species: relative to all high-density pots of both kinds, all plants were larger when grown at low densities. Further, there were differences in the strength of intraspecific versus mixed-species competition for three of the six species, with stabilizing density dependence (intra > mixed) for one native and one exotic species, and the opposite result (mixed > intra) for one exotic species. However, when net effects of all life history stages were

considered together, two native species no longer showed negative density dependence at all (*L. californica*, *V. microstachys*), and only the exotic *E. brachycarpum* showed stabilizing density dependence. Also, intraspecific and mixed-species competition became equivalent for *G. dissectum* under the net effect model. Using a similar analysis, Lortie and Turkington (2002) also saw a lack of negative density dependence in an annual plant community when 0 values were included in the data set for seeds that failed to germinate and plants that died. Since most competition studies focus on the effects of plant–plant interactions on growth stages, rather than incorporating seed and seedling stages, the prevalence and strength of negative density dependence in many competition experiments may be overestimated (Goldberg et al. 2001; Lortie and Turkington 2002).

Plants can have preferential uptake of, or differential needs for, particular forms of nutrients or water, depending on their physiological efficiencies (McKane et al. 2002). Thus, it is possible for similarly statured plants that coexist in the same pot to exploit differential resource pools (e.g., Tilman et al. 1999). We grew plants in small containers to ensure they were competing for resources, including light, water, and nutrients. We did constrain some partitioning strategies, such as rooting depth; however, these species mixes contained three species that differ in above-ground form (rosette forming forbs, erect forbs, and grasses) and differ in root morphology, both traits that are likely to affect the capture of resources. Plant roots had completely exploited pot space at the end of the experiment, and the roots of all the individuals were completely intertwined. In addition, we observed resource partitioning in time, with species emerging at significantly different rates and differing in senescence timing.

Using the evolution-based niche model, which predicts niche differentiation over time, we expected stronger negative outcomes of interspecific interactions in exotic species. Here, we assumed that these species had not been interacting long enough to coevolve. This may or may not be the case, as rapid evolutionary change has been observed in many invasive species (Carroll et al. 2007). In addition, all the exotic annuals are native to the Eurasian region. While co-occurring populations from the native range were probably not introduced to California, if coevolution of competitive ability is a species-wide,

rather than population-level phenomenon, we would not expect these exotics to have more negative interactions. Depending on the analysis method used, our data either rejected (traditional analysis, no status  $\times$  density interaction, Table 2A) or somewhat supported (net analysis, significant status  $\times$  density interaction, Table 2B) the prediction that exotic species would have stronger interactions than native species. This was the only status by density interaction seen in the entire experiment, and is due to, on average, stronger negative density dependence for both intraspecific and mixed-species competition in the three exotic species than in the three native species (though the negative density dependence in *Plantago erecta*, regardless of analysis type, should be noted). Thus, we conclude that exotic species may experience stronger negative effects of interactions than some native species, but not because adult plants interact differently. Rather, the differences in the strength of density dependence is due to stronger effects on germination and/or survival, because all the adult plants in the study showed negative density dependence at the growth stage, and differences emerge when considering the net effects of all interactions from seed to adult.

We suggest that plant competition research should continue to transition away from strict plant growth analysis toward the examination of competitive outcome that includes the analysis of the net effects of density on germination, survival, and growth. Changes in the direction and strength of competition over the life history stages of plant (e.g., Goldberg et al. 2001; Shilo-Volin et al. 2005; Miriti 2006; Schiffrers and Tielborger 2006; Lortie and Turkington 2008) are important (Brooker et al. 2005) to document to assess the importance of competition on community composition. Different life history stages could have greater competitive consequences in different systems, or when species co-occur with different neighbors. Focusing on interactions that only affect plant growth could over- or underestimate the strength of interactions between species. For five of the six species in this study, the net effects of competition fit the predictions of neutral theory: intra- and interspecific competitions were equivalent. Measuring the strength of plant–plant interactions at multiple life history stages revealed that factors other than negative density dependence are important in annual plant communities, and these analyses may change conclusions about the importance

of niche evolution in structuring annual grassland communities.

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